Cell, Vol. 121, 163–166, April 22, 2005, Copyright ©2005 by Elsevier Inc. DOI 10.1016/j.cell.2005.04.007

Minireview

Chemicals Call Bacteria, and A New Membrane Protein Machine Answers

Kevan M. Shokat[1,2,*]
[1]Department of Cellular and Molecular Pharmacology
University of California, San Francisco
San Francisco, California 94143
[2]Department of Chemistry
University of California, Berkeley
Berkeley, California 94702

A major challenge in biology is the discovery of the processes controlled by the roughly one-third of genes with no known function. One approach being explored to address this problem is the use of small molecules in conjunction with genetics—chemical genetics. A short review of this field is provided as an introduction to a series of papers in this issue of *Cell* in which a new type of chemical genetics revealed the function of a new outer membrane protein complex in bacteria.

Genetics is the dominant tool and paradigm for understanding biology. However, there are limitations to the power of genetics. Even though *E. coli* has been genetically manipulatable for over 50 years, about one-third of *E. coli* genes have no known function. Why have mutations in these genes failed to provide us with a clue as to their function? Do these genes encode missing pieces of known cellular machinery, or do some work together with other genes of unknown function in yet undiscovered molecular machines? The field of chemical biology is playing an increasingly prominent role in biology. Here I will attempt to motivate the need for developing approaches that utilize both chemical and genetic tools for studying biological systems. I will introduce the four typical "formats" for so-called chemical genetic experiments in the context of traditional genetics and pharmacology. With this as a background, I will summarize the findings described in two articles, in this issue of *Cell*, that use a new format for chemical genetics to probe antibiotic resistance and membrane biogenesis in *E. coli*, revealing a new outer membrane (OM) machine composed of four proteins of previously unknown function (Ruiz et al., 2005; Wu et al., 2005).

In the sections below I will attempt to define the two pillars of chemical genetic methods: genetics and pharmacology.

Genetics

Specific mutations can be introduced into cells or organisms providing a highly specific perturbation to complex systems. However, perturbations made to DNA or RNA are translated slowly into changes at the protein level (days for RNAi and weeks in the case of mouse genetics). Rapidly evolvable systems that can adapt to compensate for missing components, such as signal-transduction pathways, can be difficult to study without the means to rapidly perturb the system. Genetics is specific but perturbs the system slowly—allowing the system to compensate.

Pharmacology

Pharmacological agents would appear to be an ideal complement to the shortfalls of genetic tools. Highly selective and potent inhibitors of single proteins are powerful tools for complementing genetic studies because they allow the study of essential proteins and they inhibit their targets rapidly and reversibly (seconds to minutes). The limitation of pharmacology is the relatively small number of molecules with optimal properties of unique specificity and potency compared to the diversity of potential target proteins in biology. Advances in organic synthesis, structural biology, and high-throughput screening have greatly accelerated the process of small molecule tool and drug discovery, yet few such molecules have specificity that approaches that of genetics. Small molecules act rapidly but are often pleiotropic.

Chemical Genetics

Chemical diversity is at the heart of both genetics and pharmacology. Changes to DNA or small molecule structure control biological function. In the case of chemistry, we refer to the diversity of structures. In the case of genetics, we refer to sequence variation. Hence, diversity serves as a common benchmark for classifying chemical and genetic methods to study biology (Figure 1). The intersection points of chemical or genetic approaches categorize chemical genetic approaches for probing biology. In the first column of Figure 1, traditional genetics can be categorized as reverse genetics (Figure 1D) or forward genetics (Figure 1G) depending on whether a directed mutation is made or whether mutants are obtained by selections or screens. Similarly, pharmacology typically utilizes a small number of known bioactive small molecules to perturb a biological system (Figure 1B).

With these genetic and pharmacological approaches as benchmarks, we can focus on the classification of chemical genetic approaches to the study of biology. If a complex set of small molecules are screened in order to identify new pharmacological agents, the chemicals used to perturb the biological system can be viewed as the functional equivalent of point mutations. Thus, screening through a library of small molecules can be thought of as a forward chemical genetic screen (Figure 1C). Monastrol, an inhibitor of the mitotic kinesin motor, Eg5, was discovered in this assay format (Mayer et al., 1999). Small molecules that induce stem-cell differentiation to cardiomyocytes have been discovered in the same format (Wu et al., 2004). When this same chemical diversity is applied to a screen in which a "normal" and a "mutant" cell are screened in parallel to search for a mutant-specific inhibitor, one can envision a screen for anticancer agents. Screening compound libraries in the NCI-60 cancer cell line panel is an example of this chemical genetic format (Figure 1F) (Holbeck, 2004). In these two formats of chemical genetic experiments, the challenge is the production of large numbers of potentially biologically active small molecules and the dis-

*Correspondence: shokat@cmp.ucsf.edu

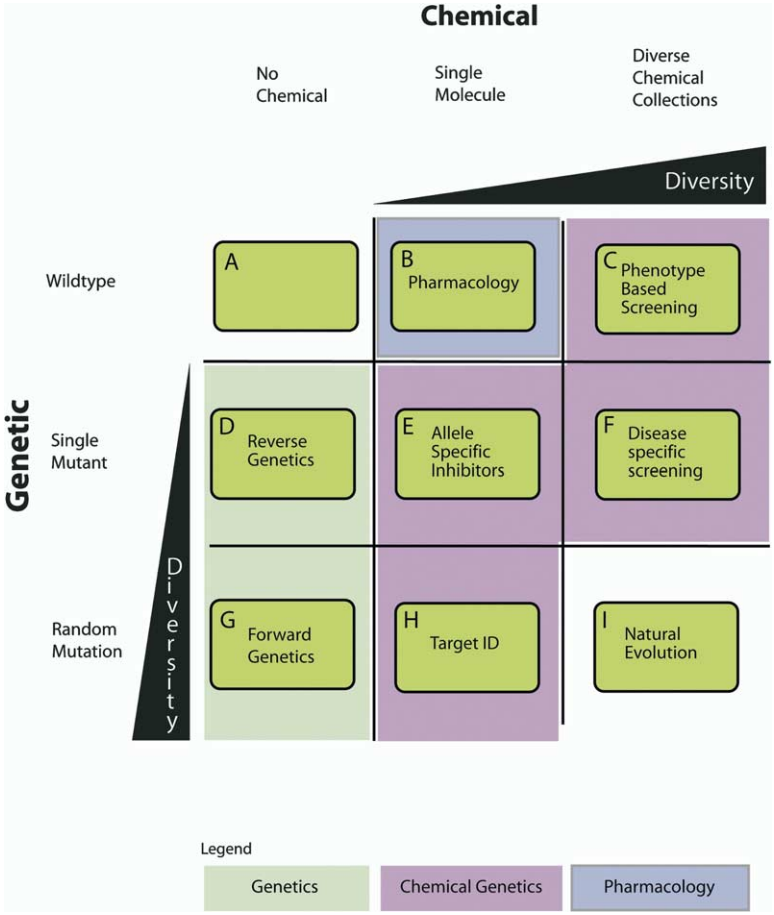

Figure 1. Classification of Chemical and Genetic Methods to Study Biology

covery of the cellular targets of "hit" molecules (Burdine and Kodadek, 2004). The challenge of small molecule target identification points out a key distinction between forward chemical genetic and forward genetic screens: the mutation can easily be recovered afterwards whereas the small molecule target cannot.

A more hypothesis-based experimental format for chemical genetic experiments has also become quite useful. If information about a protein in the pathway or process of interest is known, a more targeted approach can be used to discover selective small molecules. For example, if a specific protein kinase is implicated in a given pathway, a highly specific small molecule inhibitor for the protein kinase of interest can be readily generated using the chemical genetic format in Figure 1E (Bishop et al., 2000b). In this experiment, a point mutation is made in the ATP binding pocket of interest, rendering only this mutant kinase sensitive to a designed small molecule inhibitor that does not inhibit any wild-type protein kinases (an allele-specific inhibitor). This form of reverse chemical genetics is powerful in terms of the ability to target a single enzyme in a large family even if the active site is highly conserved, precluding use of traditional medicinal chemistry to develop a highly specific inhibitor (Bishop et al., 2000a). In this format the target is thus under genetic control providing high specificity and pharmacological control conferring rapid regulation.

The issue of the true molecular target(s) of a small molecule is a recurring challenge to the field of chemical biology as well as pharmacology. Without real understanding of the target, it is difficult to integrate results produced by chemicals into cellular pathways and processes. One of the most exciting areas of chemical genetics is the combination of pharmacology and forward genetic screens to identify the cellular targets of active small molecules (Figure 1H). A flurry of papers have applied various formats to identify mutants that are hypersensitive to pharmacologically active small molecules using haploinsufficiency or gene-deletion collections (Deutschbauer et al., 2005; Parsons et al., 2004, Lum et al., 2004).

Enter Kahne and Silhavy, whose labs have been searching for the specific cellular target(s) of a very structurally complex natural product, vancomycin (Figure 2). This antibiotic is chemically complex with a binding site for a dipeptide component of the bacterial cell wall as well as sugars that target another class of bacterial cell wall components, glycosyl transferases. In fact, its many characterized biochemical activities have led to conflicting hypotheses about the "real" target of the antibiotic. In a classic pharmacology-based effort to separate the activities of vancomycin, synthetic versions of vancomycin, like chlorobiphenyl vancomycin (CBP-vanco), were made in order to damage some properties (dipeptide binding) and preserve

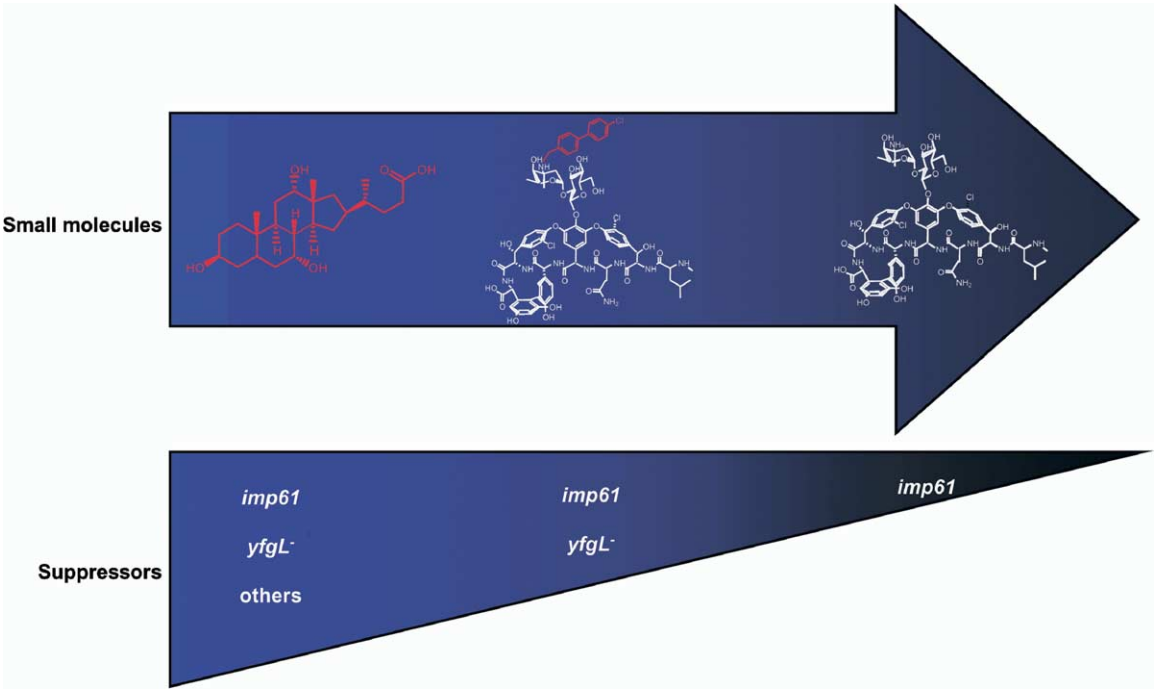

Figure 2. Continuum of Outer Membrane Mutants that Suppress the Toxicity of Small Molecules

Bile salts (top left) at one end of the continuum are suppressed by a variety of outer membrane mutants (bottom left). Vancomycin (top right) at the other end of the continuum is only suppressed by a mutation that also suppresses sensitivity to compounds to the left. CBP-vanco and yfgL lie in the middle.

or enhance others (glycosyl transferase inhibition) (Ge et al., 1999). As a test of the formal understanding of the mechanism of these designer vancomycin analogs, Kahne and Silhavy used each as the basis for a forward genetic screen for mutants that could resist the antibiotic action of CBP-vanco, hoping to identify mutations in a specific enzyme target of this antibiotic (Eggert et al., 2001). The initial goal was to resolve a controversy about the mechanism of action of vancomycin and several vancomycin analogs—do they kill bacteria by inhibition of glycosyltransferases or by binding to peptidoglycan intermediates in the membrane?

When cells were challenged with CBP-vanco, the chemical conditionality screen retrieved only mutations in an unannotated gene, *yfgL* (Ruiz et al., 2005 [this issue of *Cell*]). Prior to this screen for chemically conditional growth, no forward genetic selection or screen had identified mutations in this gene. How had a chemical genetic screen retrieved a mutant that could not be identified by genetics alone? To answer this question, Kahne and Silhavy expanded the chemical genetic screen to include a series of drug controls including vancomycin itself and molecules, such as bile salts (the red molecule in Figure 2), generally toxic to bacteria that are not structurally or functionally related to CBP-vanco. These studies showed that YfgL is *not* the actual target of CBP-vancomycin, but that *yfgL* lies on a continuum of mutations that can suppress the effects of various antibiotics. This continuum of sensitivity of the various mutant strains is only revealed by the use of structurally diverse molecules—provided by chemistry (Figure 2).

The first big "take-home message" from the chemical genetic screening perspective is that compounds of different structure and target class (vancomycin derivatives versus bile salts) can classify mutant strains according to their ability to be toxic. Without the compounds, these mutants would all be characterized as having a "leaky" membrane revealing little about their function or differential effects.

In the second paper in the series (Wu et al., 2005 [this issue of *Cell*]), the authors turned to answer the question why had the screen returned molecules in the OM of bacteria? To answer this question, they used the single gene, *yfgL*, discovered in the chemical genetic screen as a foothold into studying the OM function of YfgL. Through immunoprecipitation studies, YfgL was found to be part of a multiprotein complex in the OM that is essential for biogenesis of the OM. Moreover, this protein complex is composed of the products of three other genes of unknown function, *yaeT*, *yfiO*, and *nlpB*.

The second lesson from this study is the finding that chemical genetics has led to the identification of four proteins of unknown function operating together in a complex, a completely unknown OM machine. This suggests the exciting possibility that uncovering the function of unknown proteins may lead to entirely new cellular machines for study. Kahne and Silhavy point out some of the most interesting aspects of the function of this new complex in the context of OM biogenesis. The OM is an organelle that assembles outside the bacterial cell wall (the peptidoglycan layer)—and thus assembles in the absence of any discernible energy

source (ATP). They show that perturbing or removing one of the proteins perturbs the ability of the complex to assemble proteins in the OM. A speculative model is that the cell carefully balances the assembly of the protein and lipid components of the OM to precisely control the barrier function of this organelle. An obvious way for the cell to tightly couple expression of multiple genes is to regulate their expression by the same transcription factor. In fact, Carol Gross's lab has recently found that the genes encoding components of the same OM machine are in the sigmaE regulon, which is necessary for responding to cell envelope stress (C. Gross, personal Communication).

What lessons do these papers provide for future chemical genetic studies? A particularly surprising lesson to me was the utility of a molecule to probe some aspect of cell function other than the actual "target" of the small molecule, i.e., the enzyme active site. Traditional pharmacology teaches us to think of inhibitors as binding to an active site, lock and key, but we forget that molecules must pass through cellular membranes and may do so through interaction with specific protein or lipid components. Thus small molecules can be used to probe these membrane-proximal events and give insight into machines in the membrane that may have eluded study with genetics alone.

Finally, biology is not simply DNA-RNA-protein, but small molecules are intimate components and substrates for natural evolution (Figure 1I). Thus, many of the proteins and molecular machines we study in biology are designed to be regulated by naturally occurring small molecules (nucleotides, cofactors, lipids, etc.). Therefore it stands to reason that we must use small molecule tools to appropriately and completely study the machines in order to better understand biology (Clardy and Walsh, 2004).

Acknowledgments

I apologize for not citing many groundbreaking papers in chemical biology in this short review.

Selected Reading

Bishop, A., Buzko, O., Heyeck-Dumas, S., Jung, I., Kraybill, B., Liu, Y., Shah, K., Ulrich, S., Witucki, L., Yang, F., et al. (2000a). Annu. Rev. Biophys. Biomol. Struct. *29*, 577–606.

Bishop, A.C., Ubersax, J.A., Petsch, D.T., Matheos, D.P., Gray, N.S., Blethrow, J., Shimizu, E., Tsien, J.Z., Schultz, P.G., Rose, M.D., et al. (2000b). Nature *407*, 395–401.

Burdine, L., and Kodadek, T. (2004). Chem. Biol. *11*, 593–597.

Clardy, J., and Walsh, C. (2004). Nature *432*, 829–837.

Deutschbauer, A.M., Jaramillo, D.F., Proctor, M., Kumm, J., Hillenmeyer, M.E., Davis, R.W., Nislow, C., and Giaever, G. (2005). Genetics *432*. Published online February 16, 2005. 10.1534/genetics.104.036871

Eggert, U., Ruiz, N., Falcone, B., Branstrom, A., Goldman, R., Silhavy, T., and Kahne, D. (2001). Science *294*, 361–364.

Ge, M., Chen, Z., Onishi, H., Kohler, J., Silver, L., Kerns, R., Fukazawa, S., Thompson, C., and Kahne, D. (1999). Science *284*, 507–511.

Holbeck, S.L. (2004). Eur. J. Cancer *40*, 785–793.

Lum, P.Y., Armour, C.D., Stepaniants, S.B., Cavet, G., Wolf, M.K., Butler, J.S., Hinshaw, J.C., Garnier, P., Prestwich, G.D., Leonardson, A., et al. (2004). Cell *116*, 121–137.

Mayer, T.U., Kapoor, T.M., Haggarty, S.J., King, R.W., Schreiber, S.L., and Mitchison, T.J. (1999). Science *286*, 971–974.

Parsons, A.B., Brost, R.L., Ding, H.M., Li, Z.J., Zhang, C.Y., Sheikh, B., Brown, G.W., Kane, P.M., Hughes, T.R., and Boone, C. (2004). Nat. Biotechnol. *22*, 62–69.

Ruiz, N., Falcone, B., Kahne, D., and Silhavy, T.J. (2005). Cell *121*, this issue, 307–317.

Wu, T., Malinverni, J., Ruiz, N., Kim, S., Silhavy, T.J., and Kahne, D. (2005). Cell *121*, this issue, 235–245.

Wu, X., Ding, S., Ding, Q., Gray, N.S., and Schultz, P.G. (2004). J. Am. Chem. Soc. *126*, 1590–1591.